May 1997

DCIEM No. 97-TM-28

RISK ASSESSMENT OF BACTERIOLOGIC HEALTH HAZARDS IN THE HELMET BLADDER COMPONENT OF A PRESSURE BREATHING FOR G (PBG) SYSTEM

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Executive Summary

One of the components of the experimental development G protection ensemble known as STING (Sustained Tolerance to INcreased G) is a breathing loop consisting of a pressurized oxygen supply, regulator, face mask, and an inflatable helmet bladder. The bladder, located in the back of the pilot's helmet, at the occipital level of the skull, inflates during the positive pressure breathing phase of the Pressure Breathing for G (PBG) cycle. The bladder is inflated to the same pressure as the mask, thus pulling the mask tighter to the face; preventing mask leakage; and, ensuring an adequate supply of pressurized oxygen to the user. Upon cessation of PBG, the helmet bladder deflates.

The helmet bladder is effectively a `blind sac' in an otherwise open system. This fact has prompted the Aerospace Engineering Test Establishment (AETE) to express concern over the possibility that the bladder could serve as a reservoir for microbiological contaminants (bacterial or fungal). If such contaminants were present, deflation of the bladder, upon cessation of PBG, may result in contamination of the breathed oxygen. If so, this would pose a health hazard to the individual.

This study was carried out to examine the possibility that the helmet bladder could serve as a reservoir of contaminants and that, if present, such contaminants could be spread throughout the breathing loop. The study evaluated 7 helmet assemblies, with original bladders intact, under representative user and trial conditions: (a) pre-use (b) immediate post-use (c) 24 hours post-use with the breathing loop having been cleaned according to Standard Operating Procedures (SOP's), immediately after use (d) 24 hour post-use *without* SOP cleaning, immediately after use. Environmental swab samples were taken at the mask hose; occipital bladder hose; occipital bladder hose inlet port; and, directly from the inside of the MBU-20/P face mask. The samples were cultured on a general purpose culture media for aerobic bacteria and any resulting microbiological growth was enumerated as Colony Forming Units (CFU).

Results of the trial indicated, that under the conditions in which the STING oxygen breathing system was tested, contamination of the breathing system should not be a concern and thus the system does not appear to pose a health or safety risk to the user. Where positive microbiological cultures were detected, these were limited primarily to the immediate post-use condition and the 24 hour post-use condition *without* cleaning. Not surprisingly, the face mask showed the highest degree of contamination immediately post-use. The widest distribution of positive cultures (albeit low in number) occurred in the 24 hour

post-use condition *without* cleaning. This was the only condition in which a significant positive culture was detected in a helmet bladder. While it occurred in only one out of seven bladders, it nevertheless indicates that the `closed' bladder can sustain microbiological growth, given the right conditions. The absence of positive bacterial cultures in the 24 hour post-use condition, where Cleaning SOPs had been implemented immediately after use, indicates both the importance and effectiveness of the post-use Cleaning SOPs. Equally important is the regular and routine physical inspection of all breathing loop components to ensure the absence of cracks, holes, and areas of weakness. From a microbiological perspective, this is particularly important for `closed' components such as the helmet bladder since cracks and holes could serve as contaminant points of entry to the bladder. Should microbiological contaminants gain entry, the closed bladder environment would be conducive to microbiological growth. While the already-established Cleaning SOPs appear to be effective, it should be noted that the currently used antibacterial Savlon™ has only limited effectiveness against viruses and fungi. The effectiveness of the Cleaning SOPs could undoubtedly be improved by replacing Savlon™ with an agent such as Virkon™ which is already in use in the CF diving community.

DCIEM TECHNICAL MEMORANDUM 97-TM-28

3776-6cb14 (OMS)

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References

A. DPAS D6002-02283 (STING).

B. LSEG Tasking, (Jan. 1996).

C. Health Protection and Promotion Act, (R.R.O. 1990, Reg. 562).

D. CFTO C-22-010-026/MF-000. Airborne Oxygen Masks, (1986-10-14).

E. Zeneca Pharmaceuticals Inc. Chlorohexidine Gluconate (Savlon™) Product Information, (Oct 1996).

F. Normoyle-Elke, H. Did You Know the Might of the Microorganism? Can. J. Med. Tech. 55: 242-244, (1993).

G. Hayes, J. S., Soule, B., Klaas, S., Weisfield, A. S. and Tilton, R. C. (eds) Clinical and Pathogenic Microbiology. C.V. Moseby Co, St. Louis, pp. 67-81, (1987).

H. Virkon™, The Ultimate Virucidal Disinfectant. Antec International. Chilton Industrial Estate. Sudbury, England.

Introduction

1. The breathing loop component of the experimental development, Sustained Tolerance to Increased G (STING) system is composed of a pressurized oxygen supply, MBU-20/P regulator, face mask, and an inflatable helmet bladder. The bladder, located in the back of the pilot's helmet, at the occipital level of the skull, inflates during the positive pressure breathing phase of the Pressure Breathing for G (PBG) cycle. The bladder is inflated to the same pressure as the mask, thus pulling the mask tighter to the face, preventing mask leakage and ensuring an adequate supply of pressurized oxygen to the user. Upon cessation of PBG, the helmet bladder deflates.

2. The breathing loop is an open system, oxygen pressure and flow being governed by the MBU-20/P regulator. Activation of the positive pressure

2. The breathing loop is an open system, oxygen pressure and flow being governed by the MBU-20/P regulator. Activation of the positive pressure breathing mode (i.e. the PBG cycle) automatically inflates the occipital helmet bladder, through a connecting occipital bladder hose, at the same time as pressurized oxygen is provided to the pilot through the face mask. As this is an open system, and the only purpose of the bladder is to assist in sealing the face mask, cessation of positive pressure breathing results in deflation of the bladder through the occipital bladder hose, to the face mask, and out the exhalation port.

3. The oxygen that is released from the bladder passes through the face mask and thus there is a potential that the oxygen from the bladder may be inhaled by the individual. While the quality of the breathing oxygen *per se* is not of concern (i.e. the oxygen is stored in a sealed container and has been tested and certified for purity) the fact that the bladder is a `blind sac' means that any contaminants within it could be trapped and could be flushed out during the deflation phase of the PBG cycle. If such contaminants were microbiological in nature (e.g. bacterial, fungal, viral) their flushing into the respiratory tract could pose a health hazard risk to the individual.

4. Recognizing the fact that the helmet bladder may act as a reservoir for contaminants, the Aerospace Engineering Test Establishment (AETE) tasked DCIEM to examine this possibility and assess the potential for any health hazard risk (Ref. B). The Health Hazards Group (HHG) of the Operational Medicine Section carried out this task on behalf of the Life Support Equipment Group of the Aerospace Physiology Section.

Objective

5. The aim of this study was to test for the presence of bacterial contaminants in a selection of helmet bladders and interconnected accessories of the STING PBG system; and, if such contaminants were present, to assess any potential health hazard risk to a pilot, breathing from the STING oxygen system in both normal and PBG modes (Refs. A & B).

Methods

Sampling Protocol

6. In order for the results to have significant meaning, the testing protocol was designed to evaluate the entire helmet assembly under representative trial and user conditions. A representative sampling of seven helmets, with original bladders intact, all of which had been used extensively at DCIEM over the preceding six months, were tested under four conditions. Condition No. 1 (Pre-Test) evaluated helmets prior to use to provide a baseline comparison as well as

to test for the presence of any contamination that could have arisen during short term storage. Condition No. 2 (Post-Use) evaluated helmets immediately after the STING oxygen system was used in the normal and PBG mode to determine the extent of any immediate contamination. Condition No. 3 (Post-Use *Clean*) evaluated helmets 24 hours after use where routine Cleaning Standard Operating Procedures (SOP's) had been implemented immediately after use. This condition, relative to Condition No. 4, permitted an assessment of the effectiveness of Cleaning SOP's. Condition No. 4 (24 hr Post-Use) evaluated helmets 24 hours after use without any post-use cleaning. All helmets were worn by the same subject and adjusted for adequate fitting prior to each trial.

7. In order to adequately assess the potential contamination of each helmet assembly during use in normal and PBG modes, and to be able to relate the information gained to operational conditions, each helmet was worn for 10 min 30 sec during which a simulated STING PBG cycle was repeated three times. Each cycle comprised 3 min breathing oxygen in the normal mode at a pressure of 2 mm Hg and 30 sec breathing oxygen in the PBG mode at a pressure of 30 mm Hg. Repeated cycles were used to ensure inflation and deflation.

Sampling and Analysis Procedure

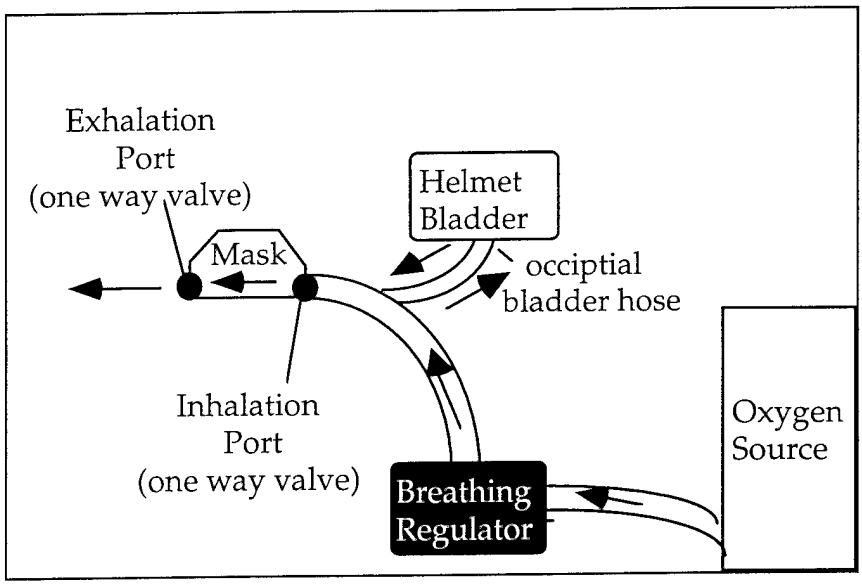
8. The presence of bacterial contamination in the STING breathing assembly was assessed under each of the four testing conditions. Duplicate swab samples were taken asceptically from the mask hose; occipital bladder hose and hose inlet port; and, directly from the inside of the MBU-20/P face mask. The swabs were then placed in sterile containers and, upon completion of each protocol, all swabs were delivered to the Ontario Ministry of Health Laboratory (Toronto) for culture.

9. The culture procedure involved placing each swab in 10.0 ml of Trypticase Soy Broth diluent and vortexing to extract any biological material. After removing the swab, the material was centrifuged, washed, and resuspended in 10.0 ml of the same diluent. Cultures were then prepared by withdrawing 1.0 ml of the suspension and plating it in a petrie dish on a Bacto Plate Count Agar (Difco Tryptone Glucose Yeast Agar). Plates were incubated at 37^{o} C for 24 hours and then examined for growth. Where growth was detected, i.e. Colony Forming Units (CFUs), these were counted and recorded. Each CFU equated to 10 colonies/swab (i.e. based on the 1/10 dilution of the procedure). Where no growth was detected, a value of <10 CFU/swab was assigned. This method of enumeration and scoring was consistent with Ontario Ministry of Health regulations within the Health Protection and Promotion Act which states, in part, that "...multi-service articles shall not exceed 100 bacterial colonies after cleaning and sanitizing and prior to re-use" (Ref. C).

Safety Considerations

10. For reasons of safety, and given the fact that the potential risk of bacterial exposure was not known prior to commencement of the study, it was deemed prudent to assess all Pre-Test results, prior to carrying out the PBG trials. This was done and all Pre-Test results were within acceptable limits.

Figure 1
Configuration of Current STING PBG Oxygen System
Arrows indicate directional flow of oxygen.



Results and Discussion

11. While the study focused primarily on an assessment of the helmet *bladder* as a potential source of bacterial contaminants, the test protocol was designed to evaluate the entire STING breathing loop assembly during normal and PBG use. The breathing loop assembly is effectively an open system and thus contamination could originate in, and spread to, any portion of the assembly. Consequently, it was necessary to evaluate all portions of the system and not simply restrict the assessment to the occipital bladder alone. As the objective of the trial was to assess the potential risk to a user of the STING oxygen breathing system it was also deemed prudent to design the protocol to reflect conditions that represent normal user or unit circumstances. Thus, the trial included assessing the breathing loop assembly after short term storage; after immediate use; and, after 24 hours use (a) where the assembly had been left uncleaned, and (b) where the assembly had been cleaned according to SOP's, immediately after use.

12. The Pre-Test protocol (Condition #1) was performed for two purposes: (a) as a safety precaution to ensure that all helmet breathing assemblies would not compromise the health and safety of the user during further testing, and (b) to provide an indication of the effects of short term storage. The results (Table 1) showed the level of contamination to be < 10 CFU/swab for all helmets. This indicated that helmets were not contaminated during short term storage (<10 CFU/swab equates to zero colony growth). The results of the Pre-Test also provided baseline data against which to compare results obtained after the STING oxygen system had been used.

13. During the trial, the STING PBG system was tested in both the normal and PBG modes of operation as described under **Methods** (para. 7). Each normal/PBG mode comprised one cycle and each cycle was repeated three times to simulate the repetitive engagement and disengagement of PBG. As depicted in Fig. 1, the helmet bladder and mask components of the STING PBG system are supplied with oxygen from the same regulator and by way of a branched tubing system. When PBG is initiated, oxygen flows into the bladder and the bladder inflates; when PBG ceases, the bladder deflates and the contents within the bladder pass into the face mask and then to the ambient environment via the mask exhalation valve. During the bladder deflation phase, 'bladder oxygen' in the face mask may be breathed.

14. As noted in the **Introduction**, the quality of the breathing oxygen *per se* is not of concern since it is stored in a sealed container and has been tested and certified for purity. It is the 'blind sac' nature of the bladder and the flushing out of its contents which could result in contaminants being trapped within and then expelled in the oxygen stream, into the face mask, where they could potentially be breathed.

15. Sampling of the STING PBG system immediately after use (Post-Test, Condition #2) provided information on contamination arising from 'normal usage' i.e. from direct contact with normal bacterial flora on the skin and in the mouth and respiratory tract. It also provided an indication as to whether or not the helmet bladder could entrap any such contaminants arising from normal flora and established a baseline against which to examine whether or not the bladder could entrap and harbour contaminants over a 24 hour period (24 hr. Post-Use, Condition #4).

16. Results of the Post-Test protocol revealed that while the face mask could become contaminated (3/7 assemblies) none of the other components showed any evidence of contamination (Table 1). Of perhaps greater significance is the fact that even after 24 hours Post-Use, and without immediate post-use cleaning, the incidence and degree of contamination was minimal (Table 1). Nevertheless, the 24 hours Post-Use assessment did reveal that a helmet bladder could possibly

serve as a repository for some growth under these conditions (Table 1). That this possibility could arise, simply emphasizes the already recognized need for standard post-use cleaning procedures. The effectiveness of the existing Cleaning SOP's was clearly demonstrated by the results obtained under Condition No. 3 (Post-Use *Clean*) in which assemblies which had been cleaned immediately after use, were evaluated 24 hours later. Results obtained under this condition were, to all intents and purposes, identical with those obtained under the Pre-Test condition i.e. no evidence of contamination (Table 1).

17. This study, under simulated conditions of operational use, revealed no significant contamination in the helmet bladder component, nor in the interconnecting hoses of the STING PBG oxygen system. Thus, under 'normal use', the possibility that the helmet bladder can serve as a trap for contaminants and create conditions which may pose a respiratory health hazard, is remote.

18. This is not to say that the possibility would never occur since any 'blind sac' can serve as a contaminant trap and, under the right conditions of temperature and humidity; inappropriate storage; and/or inappropriate or absence of post-use cleaning, conditions could arise in which contaminant growth was favoured. Clearly, contaminants can enter and be trapped within the helmet bladder under certain circumstances (1/7 helmet assemblies; 24 hr. Post-Use *without cleaning*; Table 1). While the reasons for this single finding cannot be determined from the present test results, the finding itself serves to emphasize the importance of, and need for, routine post-use cleaning. That the current cleaning SOP's (Ref. D) are effective, was clearly shown by the results obtained under test Condition #3 (Post-Use *Clean*) where virtually no contaminant growth was detected.

19. It should be noted, however, that the growth media and the 24 hr. culture incubation period used in this study, while sufficient for detecting bacteria and yeast, do not detect viruses or all types of fungi. While this fact limits the generality of the conclusions, it does not diminish the relevance of the findings nor the conclusion that the helmet bladder should not present a bacteriologic health risk under conditions of operational use.

20. Of the two undetected microbiologic contaminants, viruses are very unlikely to present a problem at any time but fungi could present a problem if helmets and other breathing loop components were stored under temperature and humidity conditions which were conducive to fungal growth. While this is a very unlikely occurrence, recognition of the possibility for fungal growth simply underscores the importance of ensuring proper storage conditions; checking the storage conditions on a regular basis; carrying out a routine physical inspection of equipment components on a regular basis to ensure that cracks or areas of weakness etc. are not present (i.e. that could permit the entry of external

contaminants); and, adopting a policy of pre-use germicidal cleaning, after extended periods of storage.

21. While this study has shown that the current cleaning SOP's are effective for the removal of bacterial contamination, it should be noted that the current cleaning product (Savlon™) is primarily an antibacterial and has limited antifungal and antiviral properties (Ref. E). Thus, if fungal or viral contamination of equipment did occur, the current Cleaning SOP's could be expected to have limited effectiveness. This potential problem could be overcome by replacing Savlon™ with a broader spectrum germicide such as Virkon™. This product is an antibacterial, antifungal and antiviral and has been shown, through independent testing, to be compatible with rubber (Ref. H). It has been used successfully in the CF (DCIEM Experimental Diving Unit [EDU]) for sanitizing diving life-support equipment and, in this regard, has been used for the past three years, without incident.

Conclusions and Recommendations

22. Based upon the results of this study, it is concluded that bacterial contamination of the STING PBG breathing loop is unlikely to occur through normal operational use and that the helmet bladder does not serve as a 'trap' for bacterial contaminants. The equipment, therefore, does not increase the risk of exposure to a bacterial respiratory health hazard beyond that which could be expected from the normal ambient environment.

23. It is concluded that existing Post-Use Cleaning SOP's are effective in neutralizing any bacterial contaminants which may enter the breathing loop components during normal operational use. Moreover, the importance of applying these SOP's after use of the equipment, has been demonstrated.

24. It is also concluded that existing Cleaning SOPs could be made even more effective by replacing the antibacterial Savlon™ with the broader spectrum germicide, Virkon™ and it is recommended that Savlon™ be replaced with Virkon™.

Table 1 Bacterial Culture Results of STING PBG System

Test Condition	Mask Hose	Occipital Bladder Hose	Occipital Bladder	MBU-2O/P Mask	Hose Inlet Port
	CFU/swab	CFU/swab	CFU/swab	CFU/swab	CFU/swab
Condition #1 Pre-Test					
Helmet 1	<10	<10	<10	<10	10
Helmet 2	<10	<10	<10	<10	<10
Helmet 3	<10	<10	<10	<10	<10
Helmet 4	<10	<10	<10	<10	10
Helmet 5	<10	<10	<10	<10	<10
Helmet 6	<10	<10	<10	<10	10
Helmet 7	<10	<10	<10	<10	<10
Condition #2 Post-Use					
Helmet 1	<10	<10	<10	*3000	<10
Helmet 2	<10	<10	<10	*150	<10
Helmet 3	<10	<10	20	20	<10
Helmet 4	<10	<10	<10	80	<10
Helmet 5	<10	<10	<10	30	<10
Helmet 6	<10	30	<10	80	10
Helmet 7	<10	<10	<10	*220	20
Condition #3 Post-Use*Clean*					
Helmet 1	<10	10	<10	<10	<10
Helmet 2	<10	<10	<10	<10	<10
Helmet 3	<10	<10	<10	<10	10
Helmet 4	20	<10	<10	10	<10
Helmet 5	<10	<10	<10	<10	<10
Helmet 6	<10	<10	<10	<10	<10
Helmet 7	<10	<10	<10	<10	<10
Condition #4 24 hr Post-Use					
Helmet 1	<10	<10	*1400	<10	<10
Helmet 2	<10	<10	<10	<10	<10
Helmet 3	<10	40	60	<10	<10
Helmet 4	<10	10	<10	<10	<10
Helmet 5	<10	<10	10	10	<10
Helmet 6	<10	<10	<10	<10	<10
Helmet 7	10	20	<10	30	<10

* indicates results above the Health Protection and Promotion Act recommended allowable limit of 100 CFU.

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Risk assessment of bacteriologic health hazards in the helmet bladder component of a pressure breathing for G (PBG) system.

4. DESCRIPTIVE NOTES (the category of the document, e.g., technical report, technical note or memorandum. If appropriate, enter the type of report, e.g. interim, progress, summary, annual or final. Give the inclusive dates when a specific reporting period is covered.)

Technical memorandum

5. AUTHOR(S) (Last name, first name, middle initial. If military, show rank, e.g. Burns, Maj. Frank E.)
Severs, Yvonne, D. Capt
Sabiston, Brian, H. Dr

6. DOCUMENT DATE (month and year of publication of document)	7.a. NO. OF PAGES (total containing information. Include Annexes, Appendices, etc.)	7.b. NO. OF REFS. (total cited in document)
June 97		

8.a. PROJECT OR GRANT NO. (if appropriate, the applicable research and development project or grant number under which the document was written. Please specify whether project or grant)	8.b. CONTRACT NO. (if appropriate, the applicable number under which the document was written)
	n/a

9.a. ORIGINATOR'S DOCUMENT NUMBER (the official document number by which the document is identified by the originating activity. This number must be unique to this document.)	9.b. OTHER DOCUMENT NO.(S) (any other numbers which may be assigned this document either by the originator or by the sponsor.)

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One of the components of the experimental development G protection ensemble known as STING (Sustained Tolerance to INcreased G) is a breathing loop consisting of a pressurized oxygen supply, regulator, face mask, and an inflatable helmet bladder. The bladder, located in the back of the pilot's helmet, at the occipital level of the skull, inflates during the positive pressure breathing phase of the Pressure Breathing for G (PBG) cycle. The bladder is inflated to the same pressure as the mask, thus pulling the mask tighter to the face; preventing mask leakage; and, ensuring an adequate supply of pressurized oxygen to the user. Upon cessation of PBG, the helmet bladder deflat. The helmet bladder is effectively a `blind sac' in an otherwise open system. This fact has prompted the Aerospace Engineering Test Establishment (AETE) to express concern over the possibility that the bladder could serve as a reservoir for microbiological contaminants (bacterial or fungal). If such contaminants were present, deflation of the bladder, upon cessation of PBG, may result in contamination of the breathed oxygen. If so, this would pose a health hazard to the individual. This study was carried out to examine the possibility that the helmet bladder could serve as a reservoir of contaminants and that, if present, such contaminants could be spread throughout the breathing loop. The study evaluated 7 helmet assemblies, with original bladders intact, under representative user and trial conditions: (a) pre-use (b) immediate post-use (c) 24 hours post-use with the breathing loop having been cleaned according to Standard Operating Procedures (SOP's), immediately after use (d) 24 hour post-use *without* SOP cleaning, immediately after use. Environmental swab samples were taken at the mask hose; occipital bladder hose; occipital bladder hose inlet port; and, directly from the inside of the MBU-20/P face mask. The samples were cultured on a general purpose culture media for aerobic bacteria and any resulting microbiological growth was enumerated as Colony Forming Units (CFU). Results of the trial indicated, that under the conditions in which the STING oxygen breathing system was tested, contamination of the breathing system should not be a concern and thus the system does not appear to pose a health or safety risk to the user. Where positive microbiological cultures were detected, these were limited primarily to the immediate post-use condition and the 24 hour post-use condition *without* cleaning. Not surprisingly, the face mask showed the highest degree of contamination immediately post-use. The widest distribution of positive cultures (albeit low in number) occurred in the 24 hour post-use condition *without* cleaning. This was the only condition in which a significant positive culture was detected in a helmet bladder. While it occurred in only one out of seven bladders, it nevertheless indicates that the `closed' bladder can sustain microbiological growth, given the right conditions. The absence of positive bacterial cultures in the 24 hour post-use condition, where Cleaning SOPs had been implemented immediately after use, indicates both the importance and effectiveness of the post-use Cleaning SOPs. Equally important is the regular and routine physical inspection of all breathing loop components to ensure the absence of cracks, holes, and areas of weakness. From a microbiological perspective, this is particularly important for `closed' components such as the helmet bladder since cracks and holes could serve as contaminant points of entry to the bladder. Should microbiological contaminants gain entry, the closed bladder environment would be conducive to microbiological growth. While the already-established Cleaning SOPs appear to be effective, it should be noted that the currently used antibacterial Savlon^{TM} has only limited effectiveness against viruses and fungi. The effectiveness of the Cleaning SOPs could undoubtedly be improved by replacing Savlon^{TM} with an agent such as Virkon^{TM} which is already in use in the CF diving community.

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STING oxygen breathing system (Sustained Tolerance to INcreased G), Pressure Breathing for G (PBG), Helmet Bladder, bacterial contamination